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Myocardial and Splenic Catecholamine Alterations in Burn Shock

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MYOCARDIAL AND SPLENIC CATECHOLAMINE ALTERATIONS IN BURN SHOCK

by

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INTRODUCTION

In a recent symposium on burns Moncrief, [39] (1963), posed the problem: "Is burn shock a specific entity?" He discussed the question whether a patient that has sustained a thermal injury is subject to a peculiar type of shock different from that seen in other types of shock. In contrast to other forms of shock, only recently have investigators clearly defined the hemodynamic and biochemical alterations seen in burn shock. It is generally accepted that the burn shocked animal has a decreased cardiac output and blood volume. A fall in cardiac output is often seen before any measurable fall in circulating blood volume is observed. Animal studies have been reported on the tissue levels of catecholamines.

It was the purpose of this investigation to measure myocardial catecholamine levels of rats subjected to burn shock, since it was believed that depletion of myocardial catecholamine may be involved in the depression of myocardial activity reported in burn shock. Splenic catecholamines were also measured since this organ is richly innervated by the sympathetic nervous system, and could serve as an indicator of sympathetic activity in burn shock.

LITERATURE REVIEW

Wilson, et al [69] (1938) classified the burn shock syndromes into four types, in which shock was accompanied by the rapid demise of the patient. The initial type was characterized by a decrease in systemic blood pressure occurring within two hours following the burn injury. In the 2nd syndrome a hypotensive episode developed after two hours and was characterized by a rapidly falling systolic blood pressure, a deteriorating radial pulse, and increasing heart rate. The classic features of shock, thirst, pain, anxiety, paleness, cold clammy skin, decreased rectal temperature, shallow rapid repiration restlessness, and distress appeared in both the initial and secondary types. The third type of clinical shock outlined by Wilson was acute toxemia which had its onset anywhere from 5 to 50 hours after the burn injury. This group of patients was generally hyperpyrexic, but again the most distinctive sign was circulatory failure. Frequently seen was an imperceptible decline in systolic and diastolic pressure or a rising diastolic pressure, terminating in an abrupt fall as circulation finally failed. Wilson's fourth group was associated with infection and subsequent septic death.

In Wilson's first three types of burn shock or those types of shock which can arise relatively early after the initial burn trauma, scientific reports have related the etiology of this type of burn shock to neurogenic, volume depletion, and toxic causes.

NEUROGENIC THEORIES

Brown-Sequard [6] (1858) demonstrated that early burn shock was a neurogenic phenomenon. He sectioned the sciatic and crural (femoral) nerves as high as possible in the dog limb before subjecting the extremity to burn. With the elimination of sensory input Brown-Sequard could demonstrate protection of the abdominal viscera from congestion which follows burn injury. He felt that he had provided proof that in great measure the burn shock syndrome was due to nervousness reflex action.

Wilson [70] (1941) stated that the noxious nerve impulses from the injured area played a role in both initiating and perpetuating shock in burns. Following Wilson's work, Kabat, Meden and Wing [30] (1943) attempted to more clearly assess the role of the nervous system in burn shock. In cats under sodium pentobarbital anesthesia, the hind limb was burned with a bunsen flame and in half of these animals afferent impulses were prevented from reaching the central nervous system by cord transection at the level of L-1. The limbs of these animals were weighed to determine if there was any fluid accumulation. They found that the average fluid lose for the animals with intact cords was 2.5% of body weight while animals with transected cords at level of L-1 had an average loss of 1.5% body weight. Wilson also reported that cord transection at the first lumbar segment effectively prevents the initial and to some extent the sustained rise in blood pressure which resulted from thermal trauma. Since the major portion

of the sympathetic vasomotor pathway remained intact when the cord was divided at this level it was felt spinal transection must eliminate the initial rise in arterial blood pressure. Interrupting afferent nerve impulses from the burned area they presume inactivated the sympathetic and adrenal response. Cord transection also greatly affected hemoconcentration decreasing it markedly when compared to controls.

Olson and Nicheles [42] (1943) conducted a series of experiments attempting to determine why blood pressure increased in burns as opposed to a decrease noted in other forms of trauma. Utilizing both dogs and cats deeply anesthetized with sodium pentobarbital or ether, they found that denervation of the burned extremity or hypophysectomy by sectioning of the sciatic and femoral nerves were "protective" against the increase in blood pressure. Upon stimulation of the central ends of the sectioned nerves, blood pressure increased. In order to assess the role of the adrenal glands and splanchic nerves in the pressor response associated with burns, the investigators adrenalectomized and splanchectomized animals; and found that the adrenal medulla and splanchic nerves played only a minor role in the pressor response. Percy [46] (1943) in dogs concluded that the immediate post burn shock syndrome was a purely reflex phenomenon. Prinzmantal and Bergman [49] (1945) also investigated the neural component of the burn syndrome in rats and like Kabat et al [30] interrupted the spinal cord by transection to determine if interruption

of the spinal cord had an effect on the survival time. They did not find a significant increase in survival time in the transected cord animals, and it was their conclusion that some humoral factor must be involved.

VOLUME DEPLETION THEORIES

It was Baraduc's [2] classic monograph in 1862 that first described that hemoconcentration was associated with burns. Tappeiner [60] (1881) proposed the possibility that local edema and plasma loss could account for hemoconcentration and burn shock. Blalock [5] (1931) in a series of experiments to determine if volume loss could be extensive enough to produce the shock of burns demonstrated that it was quantitatively sufficient. Harkins [25] (1935) in developing the local fluid loss theory of burn shock showed that the shift of fluid into the burn site was rapid. His studies demonstrated that 40 to 75% of the total amount shifted into the burnt area in the first hour. Rossiter [51] (1943) after numerous experiments and a review of the literature concluded that in burn shock it is probable that the enormous losses of plasma from the blood can initiate shock. This factor of burn shock is one of immense importance and justifies vigorous counter-measures. Prinzmantal et al [49] (1944) concluded that the loss of fluids isn't always the cause of burn shock. They demonstrated that a rat limb burned for 10 seconds at 65°C developed considerable edema. Burning the limb at 100°C or 150°C for 15 seconds to two minutes resulted

in no visible edema, yet the rat died. Thus in their experiments the shock was not proportional to fluid loss. Dobson and Warner [15] (1957), utilizing radioactive I^{131} albumin in dogs, demonstrated that the cardiac output falls before plasma volume reduction ensues, and they concluded that local fluid loss is symptomatic of burn injury rather than etiologic of burn shock. They also reported that when fluid loss does occur rapidly in the first 10 minutes as Harkins [25] had described, but no significant depression of cardiac output is associated with this loss. This conclusion of Dobson and Warner [15] is also supported by the earlier study of Gilmore and Handford. [21] Braasch [7] (1963) suggested an interesting mechanism of volume depletion in severe thermal trauma. He found that both burns and anoxia led to a 7% increase in size of erthyrocytes. He correlated this increase in erthyrocyte volume with the reduction of cardiac output. He further demonstrated that when RBC volume is corrected by the administration of hypertonic solutions the depression in cardiac output is corrected also. A similar amount of saline was ineffective in correcting the cardiac output.

TOXIC THEORIES:

Audkakoff [64] (1876) is quoted as the first worker to have reported that blood from a burned animal was toxic to a recipient animal. In 1922 Vaccarezza concluded that a toxic substance was carried in the blood stream of burned dogs. In a series of experiments, he anastomosed vessels of burned dogs to unburned dogs.

The unburned dogs died. Wilson et al [68] (1937) was able to provide considerable evidence for a toxic theory of burn shock. They showed that edema fluid collected 48 hours after burn was toxic to the nervous and circulatory systems and caused liver degeneration. The toxic factors were further shown to be a histamine like compound. In attempting to identify a toxin, Wood [71] (1940) investigated the lymph of burned animals. He subjected an isolated frog heart to two solutions, one containing the extract of cauterized muscle and the other an extract of non-cauterized muscle. The muscle tissue was taken from the same animal as the heart utilized in the preparation. He found depression in rate and force of contraction; changes in heart rhythm were also observed. Aldrich [1] (1944) demonstrated the presence of a vasoconstrictor substance in lymph of burned animals. Basset [3] (1951) reported the presence of a cardio-depressive substance obtained from cauterized muscle which produced strong depressor effects upon myocardium. From 1940 to 1960 the investigation of toxic factors in burns was dominated by Rosenthal. [52, 53] In a review of the subject in 1959, he states:

> "The large majority of those workers in the field agree that, although fluid, colloid, and salt loss contribute greatly to death in the first 24 to 48 hours after extensive burns, when these elements were compensated for in great measure, death still occurred in a fairly high percentage of such animals or individuals. The fatal outcome could not be explained entirely by the factors mentioned; therefore, some other cause was responsible, most probably a "burn toxin."

He concludes his report by stating that a lethal substance does exist

composed primarily of peptides, polynucleotides, hexoses and pentoses; and that histamine bradykinin, and adenyl compounds are contributory to but not synonymous with the "burn toxin". Warner and Dobson [64] (1956) reported alterations in the electrocardiogram following experimentally induced thermal injury. They demonstrated with serial ECG's that the QRS complex was immediately depressed after burns and progressively declined. There is also a corresponding T wave depression and frequent pulses alternans. They felt that depression of QRS complex paralleled the decrease in cardiac output and that a myocardial toxin could be involved. Fozzard [18, 19] (1960) released his much quoted paper in which he demonstrated that the cardiac output fell well before any volume change and that pulmonary edema was easily produced by a fluid overload. This is contrary to what is seen in normal animals with fluid overload. When these animals were given digitalis with sufficient fluids the cardiac output returned to normal. Since fluids alone did not correct the cardiac output completely, he concluded that in the burn animal the heart was failing. Merriain [35] (1962) investigated myocardial function in dogs in early post burn periods. He utilized left ventricular function curves as an index of myocardial contractility and found that the ventricular function curve measured 30 minutes after injury shifted to the right and that there was no great increase in arterial pressure or change in heart rate. He felt that the possibility of a direct myocardial toxin could not be ruled out. Spector [57] (1963) working in vitro with isolated

guinea pig myocardium found that the addition of serum from the burned dog taken 30 minutes post burn did not effect substrate metabolism. There was also no evidence of oxidative phosphorylation being uncoupled. The experiment failed to show any interference with myocardial substrate utilization. Rosenthal [52] (1965)reported that a definite lethal factor could be isolated from burned skin, and he felt that this lethal factor was not histamine, adenylic compounds, brady-kinnin, or serotonin because five to ten times that amount seen in the perfusates was not lethal.

Baxter et al [4] (1966) utilizing cross circulation cross perfusion experiments demonstrated that when a normal dog was cross perfused with a burn dog 24 to 36 hours post burn; the normal dog's cardiac output was depressed from 45 to 70%. An average depression of 33% in cardiac output occurred within three to five minutes after the onset of cross perfusion. In normal dogs cross perfused with normal dogs, the cardiac output decreased in average of 15%. They postulated that the cardiac output in dogs crossed perfused with burn dogs, decrease was not due to alternations in acid base balance, pulmonary function or blood volume changes, but that this decrease seen could be due to a toxic factor. Despite the investigations cited few conclusions can be reached about role of "toxins" in the etiology of burn shock.

HEMODYNAMICS IN BURN SHOCK

Prinzmantal et al [49] (1945) attempted to delineate some of the circulatory changes seen in burn shock. They found that in animals burned at 100°C for 45 seconds that the bleeding volume was 3.2% of body weight. When burned at 100°C for two to three minutes the bleeding volume was 1.9% of body weight, while in normals the bleeding volume was 4.3% of body weight. Dobson and Warner [15] demonstrated in scalded dogs that the cardiac output decreased to one-third of the preburn levels within six minutes despite an increase of 10% in plasma volume as determined by radioactive I^{131} tagged albumin. At one hours post burn the cardiac output was similarly depressed but plasma volume had now decreased by 20%. The decrease in plasma volume lagged behind the decrease in cardiac output by approximately 15 minutes. The arterial pressure was found to increase at the onset of burn and then decreased approximately 15% within the first 15 minutes. It remained constant for the next five hours. Their conclusion was that the decrease in cardiac output and increase in arterial pressure was the result of massive vasoconstriction. Page 44-45 (1944), working with dogs demonstrated that in scald burn the intra-thoracic venous pressure increased markedly during burning. This pressure returned to the normal range within 5 minutes of the burn. He also showed that there was constriction of large and small arteries and veins after burns. Recent investigation of Michie et al [36, 37] (1964) are probably the most meaningful in defining the hemodynamic alterations encountered in burn shock. It was

their plan to define the hemodynamic alterations, utilizing modern techniques with adequate controls. In mongrel dogs under nembutal anesthesia with 50% body surface scald they found blood pressure rose to 20% above normal during burn, and fell 20 - 30% below in five hours. Heart rate usually was elevated 20% above normal. The cardiac output decreased 20% in five minutes, 60% in ten minutes, and 60 to 80% within six hours after burning. The mean circulatory time increased 60% within one hour post burn and a marked decrease in central blood volume was also seen. The right arterial pressure was found to decrease to 178% below control levels. The hematocrit in these studies increased 21% over normal, but this did not parallel the change in cardiac output. They concluded that the maintenance of a good arterial pressure with a decreasing cardiac output indicated an increased peripheral resistance, due possibly to a vascular or viscosity change. Since the cardiac output fell more rapidly than the hematocrit increased, the hemic component of the peripheral resistance alone doesn't account for the depressed cardiac output.

Moncrief [38, 39] (1965) in patients showed that the cardiac output can decrease 70% of normal in the first 24 hours after burn, and that various fluid regimens could effect this decrease in cardiac output. In control studies in dogs, he found that when peripheral resistance was calculated (as the quotient of the mean arterial pressure divided by the cardiac output considering effective venous pressure as zero) that immediately post burn (within 5 minutes) the peripheral resistance

exceeded control values by 200%. Within one hour post burn the resistance either increased or decreased not following a constant pattern. In attempting to monitor venous pressure, he found that it fluctuated between 1 and 7 mm/Hg. Moncrief admitted that the data is inaccurate due to interference of respiration. He concluded that the heart was essentially involved as a major factor in burn shock since the cardiac output decreased before the changes in the blood volume. His study also revealed that in the burned animal the heart does respond adequately to volume replacement when treated with inotropic agents.

Kaye and Mason [31] (1966) utilizing cardio-pulmonary by-pass in dogs with constant flow demonstrated that immediately post burn a cardiac output increase is observed. The right atrial pressure initially increases, and later declines; while left ventricular work decreased in the immediate post burn period. A slow increase in peripheral vascular resistance was observed, but pulmonary resistance remained stable.

CATECHOLAMINES IN BURN SHOCK

It is well known that the sympatho-adrenal axis is intimately involved in stress and trauma reactions. Goodall and Hayes [22] (1960) studied the catecholamine content of the adrenal medulla in fourteen patients who died subsequent to severe thermal trauma. Utilizing bioassy, norepinephrine by means of alteration in cat's blood pressure and epinephrine by reaction of fowls rectal cecum, they discovered that 2/3 of these patients demonstrated subnormal levels of epinephrine in the adrenal medulla. By following the urinary catecholamine output of this group it became apparent that the patients fell into three groups: (a) low to normal epinephrine output with normal epinephrine content of the gland, (b) high epinephrine output with a low adrenal gland content, (c) low epinephrine output and low adrenal gland content. The patients in group (a) had less severe burns than those in group (c) and thus possibly less stress. Goodall and Moncrief [24] (1965) reported that in thermal injury the levels of norepinephrine and epinephrine found in the urine were elevated in some cases for weeks. The urinary content of catecholamines was observed to spike approximately five days prior to death in burn patients and it was suggested that this may be a useful clinical sign of the terminal stage of shock. In 9 of 12 patients it was found that noradrenalin in the lumbar sympathetic axon and ganglion was markedly decreased, while epinephrine in these axons and ganglions was relatively constant. Moncrief [39] (1965) raised the question as to whether or not this represents a depletion of

norepinephrine from the sympathetic nerves, because most patients dying from burns are hypotensive and norepinephrine infusion is helpful in elevating blood pressure.

The post burn patient utilizes increased amounts of oxygen has been explained as reflection of greatly increased loss of water vapor in the post burn period. The energy required to vaporize and maintain this large insensible H_2O loss is one reason why more O_2 is utilized. In view of the foregoing Harrison [26] (1967) et al decided to determine if there was any ocrrelation between the magnitude of the increase in oxygen consumption, and the degree to which catecholamien excretion is elevated. To determine the catecholamine levels he used the fluorometric technique of Von Euler [16] (1965). To evaluate his results critically he plotted total free excretion of epinephrine and norepinephrine against corresponding abnormal O_2 consumption rate (abnormal being greater than $\pm$20%). It was found that the decline in catecholamine excretion appeared to precede the fall in metabolic rate by approximately one week, but their data was insufficient for critical evaluation. The correlation of total catecholamine excretion and O_2 consumption was significant $P.<o01$ and regression of the ideal curve to a metabolic rate of +20% revealed a figure for total catecholamine excretion well within the normal daily range. Harrison [27] (1967) suggested that some ill effects might result from these abnormally high blood levels of catecholamines as reflected by urinary values. In some cases as much as 400 to 500 micrograms

per day of catecholamine were found in the urine. In the hearts of some patients who manifested these extremely high catecholamine levels, occasionally subendocardial lesion similar to those seen in rats and rabbits injected with high doses of noreadrenaline (NE) were found.

MATERIALS AND METHODS

1) Surgical Procedures

White male rats weighing approximately 500 gms were anesthetized by an initial intraperitoneal injection of 40 mg/kg of sodium pentobarbital, and a surgical level of anesthesia was maintained by administering small doses of sodium pentobarbital during the course of the experiment. The hair was closely clipped from the base of the tail to the tip of the xiphoid. From a midline incision in the neck the trachea was cannulated and the right common carotid artery was catheterized. Arterial blood pressure was recorded with a 267B Sanborn pressure transducer connected to a Sanborn model 15[illegible] recorder. The arterial blood pressure catheter was maintained patent by periodic use of 3% heparinized saline. The animals were divided into two groups, one of which served as control, while the second group was subjected to a scald burn. The burn was induced by immersion of the lower one half of the body for 35 to 45 second into water heated to a temperature of 90 to 95°C. The burned animals were further divided into two groups. In one group the animals were observed until the blood pressures had declined to approximately 60/40 mm/Hq. At this level of blood pressure the experiment was terminated by rapid excision of the heart and spleen. In the second group of burned animals the experiment was terminated at random times and pressure. In all groups ventricular and splenic tissue was taken; the ventricles were quickly freed of atrial and

fat tissue. Both the ventricles and spleens were washed with 5 cc. 0.9% NACl, blotted of excess fluid, and frozen in liquid nitrogen. Samples stored at -5°C were analyzed for catecholamine content within a week following the experiment. The experiments were conducted on two rats simultaneously; one was subjected to the burn trauma while the second animal served as control. Both animals were sacrificed at the same time. In order to assess the influence of surgery and prolonged catheterization upon myocardial catecholamines, ventricular tissue was analyzed for catecholamines in a group of unanesthetized rats subjected to decapitation.

CHEMICAL ANALYSIS:

The total ventricular sample and spleen were analyzed for their content of norepinephrine and epinephrine according to the methods described by Price and Price [48]. The tissue samples were weighed on a Roller Smith Tissue Balance and placed in a conical Potter-Elvehjam tissue grinder containing 5 ml of cooled 10% trichloracitic acid. While immersed in ice water tissue was homogonzied. The homogenate was centrifuged in a refrigerated centrifuge and the supernatant removed. An equal volume of 8.2N sodium acetate was added and the ph of the solution carefully adjusted to ph 8.2 with 1M sodium carbonate. The ph was measured in Beckman Model G PH meter. The tissue extracts were eluted from a prepared alumina column, which consisted of a glass tube with diameter of 10 mm and an aperture of 5 mm with a glass wool pulg and 1 gram of

previously washed alumina was added and half filled with triple distilled H_2O. The water and subsequent washes of 8.2N sodium acetate previously adjusted to a ph 8.6 were forced through the alumina column by bulb pressure. The columns were considered ready when the acetate wash had a ph reading of 8.6. The tissue extract was then poured into the prepared columns and eluted with 10cc of .3N acetic acid. The eluate was analyzed by the trihydroxxindole method. The 10 mg of eluate was divided into three portions of 3 ml each, of which two were analyzed for norepinephrine and epinephrine and the third served as a reagent blank. After the addition of 1 ml 1 N sodium acetate buffer, the ph of each eluate fraction to be analyzed was adjusted to a ph of 6 by the drop wise addition of 1 N sodium hydroxide with 2 drops of .04% bromthymol blue serving as the indicator. The sample was treated with 0.1 ml of 0.25% potassium ferricyamide, and after five minutes 0.1 ml of freshly prepared ascorbic acid and 0.9 ml of 20% NaOH were added. After four minutes the eluate was converted to ph 5 by adding 2.0 ml of 5 N acetic acid and adjusting the volume to 10 ml with triple distilled water. At this point the samples were immediately read. The blanks were prepared in the same manner except that the ascorbic acid was added first, and thus the reaction by which the flourescent product is formed from the catecholamine was not allowed to proceed. The flourescent compounds known as the lutines were measured with a Model 4-8202 Aminco-Bowman Spectrophotofluorometer. The emission wave length was set at 510 mu and read at two wave lengths of 400 mu and 440 mu.

The use of reagent blanks may give rise to some questions in this method. Various blanks can be used. Lund [34] (1949) introduced the concept of 'faded' blanks whereby the fluorescent lutine is formed, but it is allowed to fade. Price and Price [46] described the method of reagent blanks used in this study. Ascorbic acid is added first, followed by potassium ferricynide and sodium hydroxide. In this manner epinephrine and norepinephrine are prevented from being oxidized and the flourescent lutine does not develop upon the addition of alkaline. It is true that this method gives consistently higher blank readings than do triple distilled water blanks, but it was felt that the reagent blanks were more precise and, therefore, were employed in this study.

It has been suggested that the addition of 5 ml of 5N acetic acid be omitted in the catecholamine analysis because it allows the solution to rapidly deteriorate and only accomplishes the reduction of dopa and dopamine which under normal conditions contribute only 1% of the fluorescence when present with epinephrine in equimolar concentrations. Even with this in mind, it was decided to carry out the acidification step as recommended by Price and Price since in the burned animals the concentration of amines other than norepinephrine and epinephrine is ill defined.

Calculations of NE and E are performed by comparing the fluorescence obtained per microgram of standards NE and E with the net fluorescence observed in the unknown. This yields the following

simultaneous equations which can be solved algebracially:

Equation 1: $F_{400} = mE + nNE$

Equation 2: $F_{440} = oE + pNE$

F_{400} and F_{440} are the numbers of galvonometer divisions observed during measurement of sample excitation. The m n o p are galvonometer defections for standard NE and E solutions per microgram. NE and E in the formula are NE and E concentrations in the unknown.

Table I contains data from a series of recovery studies undertaken. In these studies freshly prepared NE and E alone and in combination in ranges expected to be obtained from tissue studies were added to the extraction mixture and then subjected to analysis for catecholamine. The average recovery was 88% with a range from 83 to 97%.

TABLE No. 1

RECOVERY STUDIES

No.	SAMPLE	RECOVERY NE	ugm E	%
1	1 ugm E	0	.90	90
2	1 ugm E	0	.88	88
3	1 ugm E	-	.85	85
4	1 ugm E	-	.82	82
5	1 ugm NE	.87	.02	87
6	1 ugm NE	.91	.06	91
7	1 ugm NE	.83	.02	83
8	1 ugm NE	.89	.02	89
9	2 ugm NE	1.89	.02	94
10	2 ugm NE	1.82	.06	91
11	2 ugm NE	1.88	.06	94
12	1 ugm NE 1 ugm E	.85	.80	83
13	1 ugm NE 1 ugm E	.87	.88	87
14	2 ugm NE 1 ugm E	1.87	.69E	85
15	2 ugm NE 1 ugm E	1.89	.82E	83
16	2 ugm NE 2 ugm E	1.95	2.03	97
17	2 ugm NE 2 ugm E	1.90	1.93	95
			MEAN RECOVERY	88%

In order to assess the extraction procedure and the effect of other tissue components, catecholamines were added to normal heart before analysis. Table II indicates that the recovery of both known quantities NE and E added to tissue samples was acceptable.

TABLE NO. II

NORMAL RAT VENTRICULAR TISSUE AND EXOGENOUS CATECHOLAMINE

No.		Exogenous Catecholamine NE ugm	Exogenous Catecholamine E ugm	Recovery NE ugm	Recovery E ugm
1	Heart wt. 1.0 gm	1.0	1.0	1.79	.75
2	Heart wt. 195 gm	1.0	1.0	1.89	.89
3	Heart wt. 1.2 gm	1.0	1.0	1.93	1.10

RESULTS

In a group of 13 rats subject to scald trauma, the acute blood pressure response to immersion into water at 90 to 95°C varied as indicated by Figure 1. In some rats there were sharp increase in both heart and systemic blood pressure; while in other an initial decrease in both parameters was observed. In all animals the blood pressure and heart rate were slightly elevated above control for one to two hours after the burn.

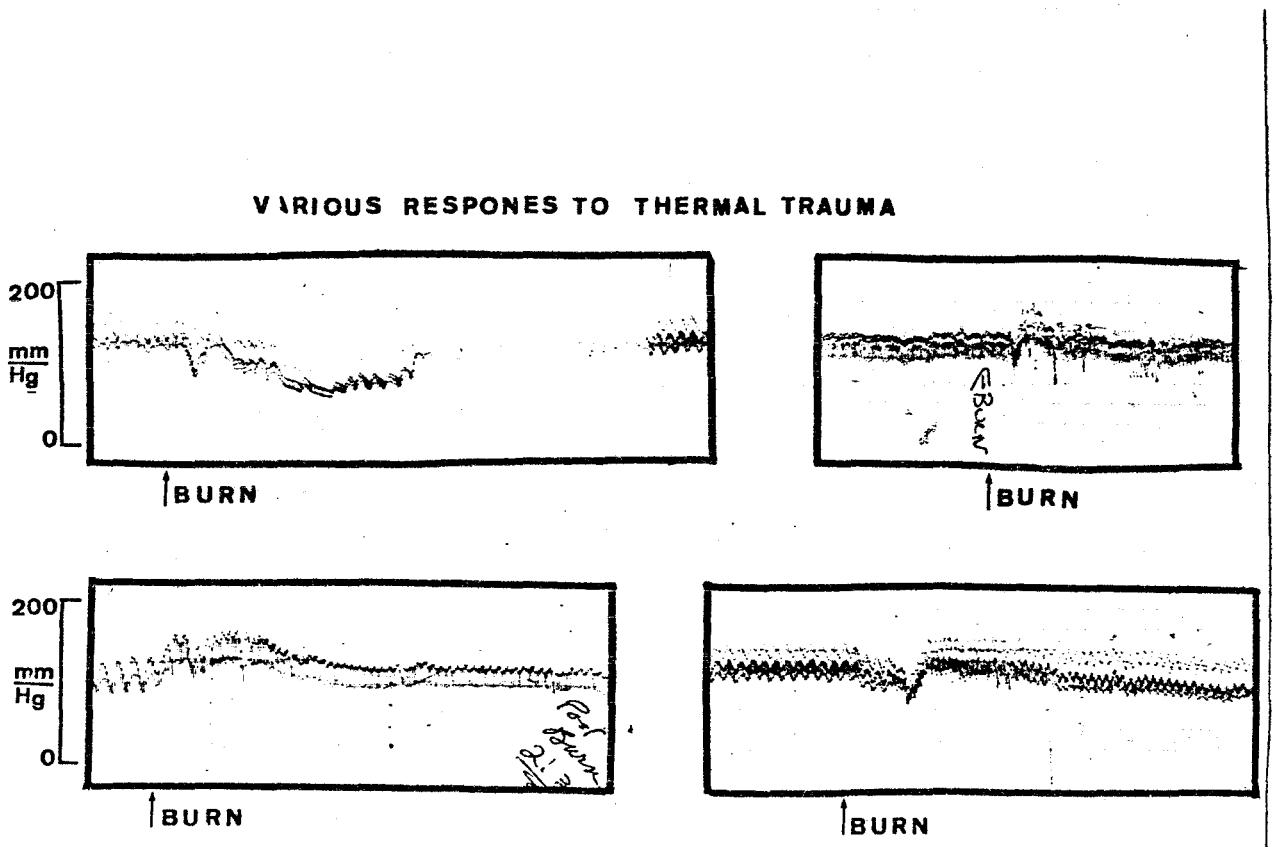
Figure II is a typical record of the systemic blood pressure from a burn shocked rat. It is noted that after the burn injury, systemic blood pressure and pulse rate are slightly elevated, and that pressure gradually declines throughout the 6 hour period. At the time the animal was sacrificed the blood pressure was approximately 60/30 mm/hq. It is felt that the animal at this time was no longer capable of compensating reached an "irreversible" stage of burn shock. In a control series of rats subjected to same surgical procedures and immersion into water at approximately 38°C, no significant alteration in blood pressure was observed for six hours.

Table III compares the ventricular myocardial content of NE and E from the control and burn shock groups. The rats subjected to thermal trauma that were sacrificed when systemic blood pressure had declined to approximately 60/40 mm/hm a significant decrease to an average of 26±.20 ugm/gm of NE ($P<.001$) was found in the burn shock group when compared with normals. Myocardial E level in rats after

burn injury rose to .24±.07 ugm/gm when compared with an average control level of 0.06±05 ugm/gm ($P < .003$). The decrease in NE was 66%, while the increase in E was four fold. The total ventricular catecholamine content declined 38% in the burn group.

Figure I

Acute Blood Pressure Responses to Scald Injury in the Rat.



⊢ = 10 seconds.

Figure II

Blood Pressure Recordings from a Rat Subjected to Thermal Injury

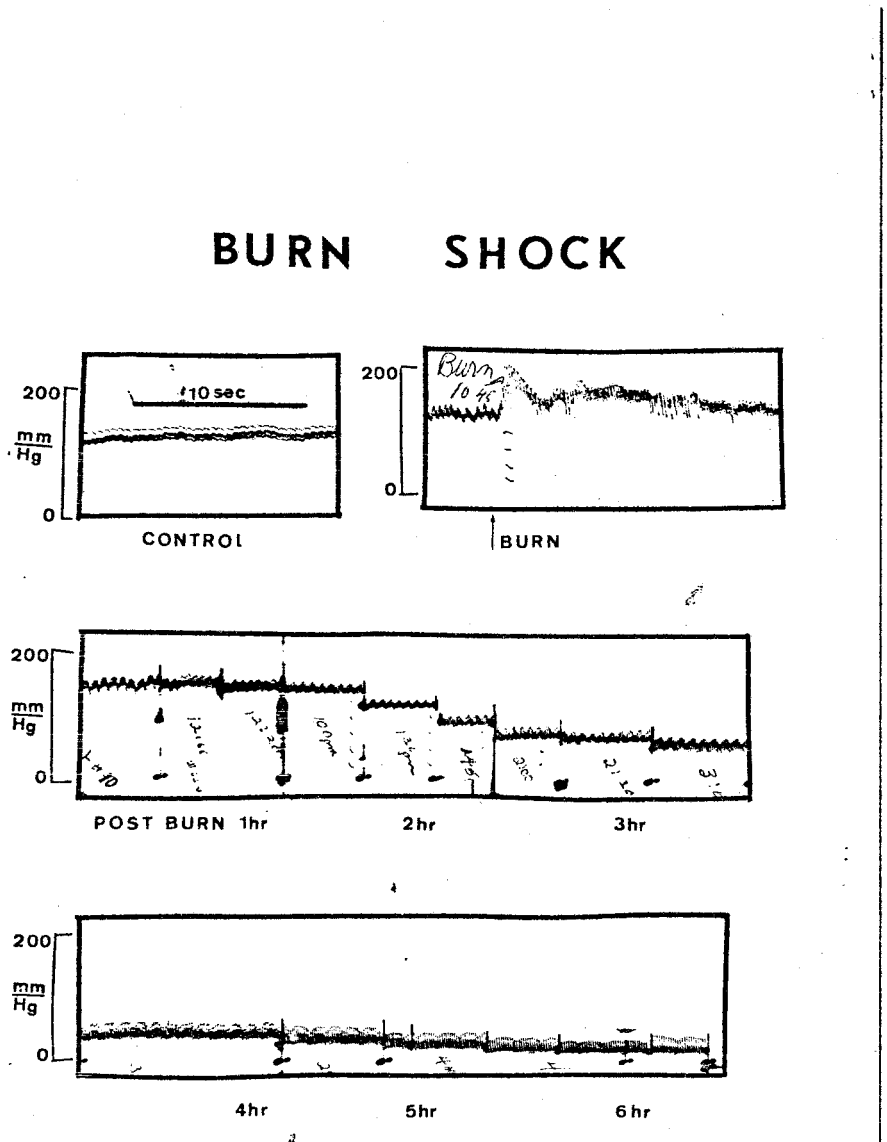


TABLE III

NOREPINEPHRINE AND EPINEPHRINE LEVELS IN THE HEART OF CONTROL AND BURN SHOCKED RATS

Controls

No.	NE ugm/gm	E ugm/gm	No.	NE ugm/gm	E ugm/gm
1	.93	.06	8	.65	.12
2	.63	.01	9	.89	.03
3	.92	.11	10	.74	.01
4	.62	.17	11	.65	.06
5	.67	.03	12	.91	.12
6	.91	.01	13	.92	.10
7	.65	.05			
			Mean ± S.D.	80.0±.12	106±.05

Burn Shock

No.	NE ugm/gm	E ugm/gm	No.	NE ugm/gm	E ugm/gm
1	.11	.21	7	.10	.18
2	.10	.25	8	.14	.25
3	.19	.24	9	.17	.39
4	.08	.30	10	.48	.31
5	.55	.21	11	.39	.18
6	.58	.22			
			Mean ± S.D.	.26±.20	24±.07
			"p" value	<.001	<.003

TABLE IV

MYOCARDIAL NOREPINEPHRINE AND EPINEPHRINE OF DECAPITATED UNANESTHETIZED RATS

No.	NE ugm/gm	E ugm/gm
1	.84	.04
2	.79	.10
3	.89	.20
4	1.1	.09
5	1.3	.07
6	.91	.12
Mean	.97	0.10

In order to assess the influence of the surgical procedure and prolonged catheterization of the carotid artery on the myocardial content of catecholamine, six unanesthetized rats were quickly decapitated and ventricular tissue was taken.

Table IV contains the levels of NE and E found in these animals. The average NE and E levels were .97 ugm/gm and .10 ugm/gm respectively. These levels did not differ significantly from those of the control group.

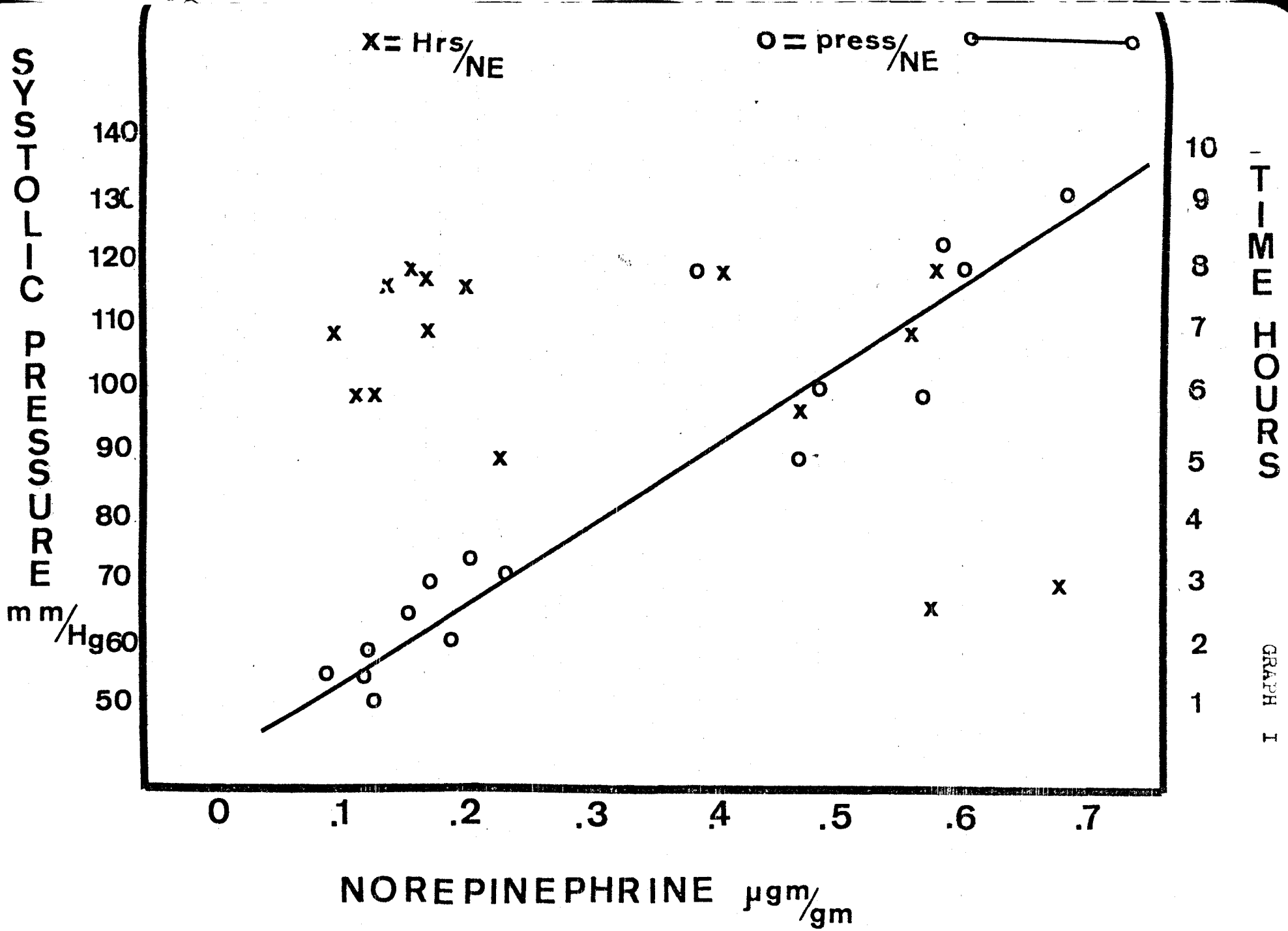
Splenic levels of catecholamines for control and burn shock animals are shown in Table V. In rats subject to burn shock the NE content .99±26 ugm/gm had increased markedly over control levels of .66±20 ugm/gm ($P < .05$). This rise in the sympathetic neurotransmitter NE in the spleen was accompanied by a fall in E from an average of .34±.12 ugm/gm in the control group to .12±.06 ugm/gm ($P < .003$) in burns shock animals. The total splenic catecholamine level rose 13% despite a decrease in E content of 250%.

An interesting correlation between systolic pressure and myocardial NE content was found in the group of rats subjected to thermal injury and sacrificed at random intervals.

TABLE V NOREPINEPHRINE AND EPINEPHRINE LEVELS IN THE SPLEEN OF CONTROL AND BURN SHOCKED RATS

No.	Weight/gms	NE ugm/gm	E ugm/gm
		CONTROL GROUP	
1	.85	.93	.22
2	.89	.34	.39
3	1.30	.70	.49
4	.88	.43	.37
5	1.20	.52	.48
6	1.12	.73	.27
7	----	.57	.18
8	1.22	.98	.54
9	.82	.47	.06
10	.98	.37	.25
11	.95	.71	.48
Mean ± S.D.	.91	.61±21	.34±12
		BURN SHOCK GROUP	
1	1.00	1.10	.20
2	.94	.70	.40
3	.86	.91	.03
4	.66	1.39	.06
5	1.24	1.18	.06
6	.96	1.22	.08
7	1.13	.95	.21
8	.83	.81	.04
9	1.09	.69	.13
Mean + S.D.	.96	.99±26	.13±.06
"P" Value		.05	.003

Graph I represents a relationship between myocardial NE content and NE content at various systolic pressures correlated with the time after injury. No statistical analysis of this data was attempted, but the data does suggest that when systolic pressure is low there is a concomitant decrease in myocardial NE content irrespective of the time interval after thermal injury.


x= Hrs/NE
o= press/NE
SYSTOLIC PRESSURE mm/Hg
140
130
120
110
100
90
80
70
60
50
0
.1
.2
.3
.4
.5
.6
.7
NOREPINEPHRINE µgm/gm
TIME HOURS
10
9
8
7
6
5
4
3
2
1

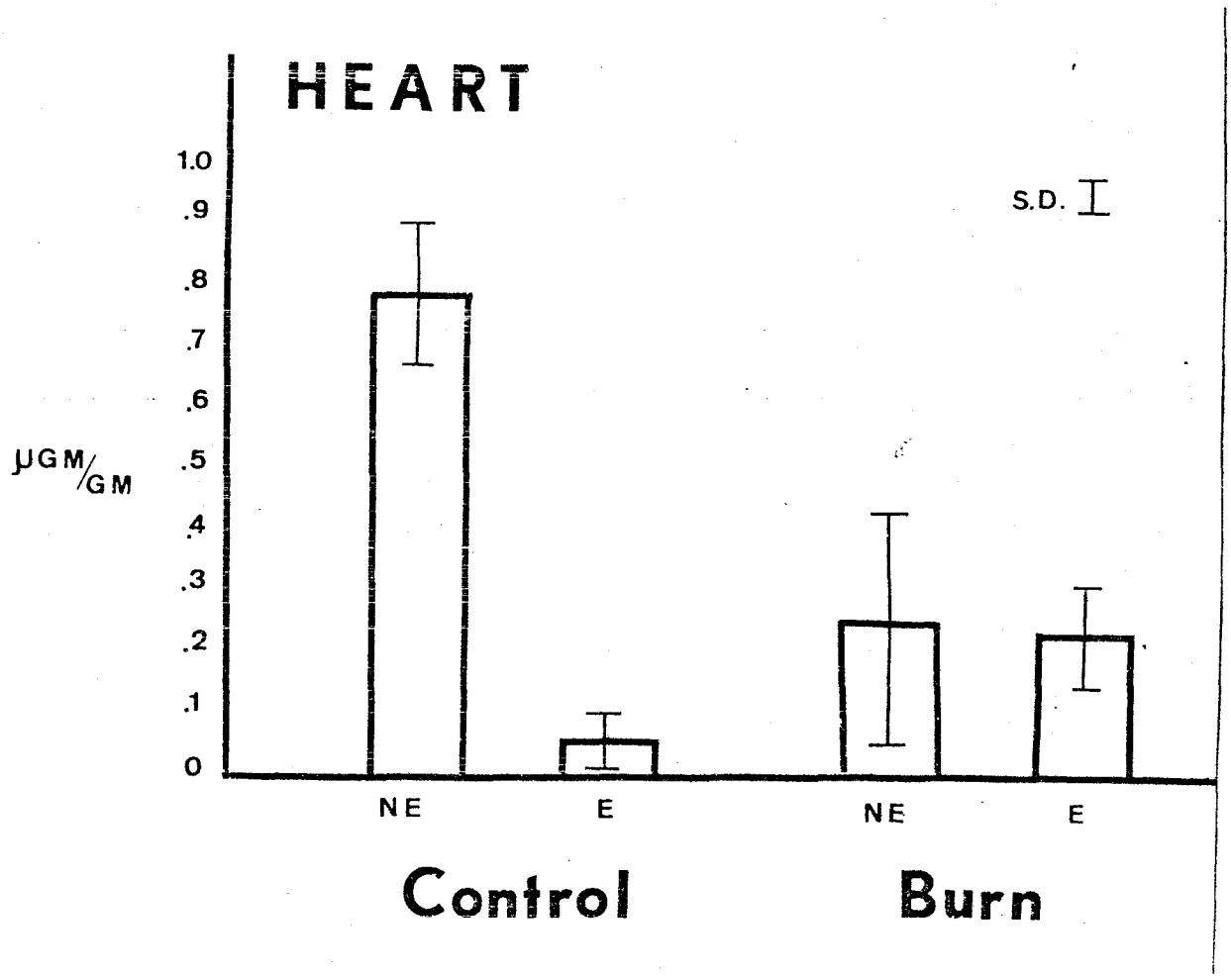
GRAPH I

DISCUSSION

The findings Harrison [26] (1967) of increased excretion of NE and E in patients and experimental animals in the post burn period strongly resembles the finding of Chidsey et al [8] (1965) and Tomonatsu et al [61] (1963) in congestive heart failure. Tomonatsu in Japan was the first to observe that there was a marked increase in both urinary NE and E in experimental and clinical congestive heart failure. Chidsey et al in this country working with patients with congestive heart failure reported an increased urinary catecholamine excretion, which they found to be primarily NE. They stated that the increase in excretion of catecholamine reflected an increase circulating blood catecholamine concentration. It is interesting to note that the excretory product in burned patients was NE and not vanilmandelic acid, the normal metabolic end product. Chidsey also demonstrated a correlation between urinary and myocardial catecholamine levels. The myocardial sample utilized in their clinical study was atrial appendage removed at the time of valvular surgery. They found that when urinary excretion was high (greater than 22.5 ugm/day) the atrial appendage contained little NE. Following quickly upon this clinical study, Chidsey et al [9] (1965) and Spann and Chidsey [55] (1966) working with dogs and guinea pigs with experimentally induced heart failure found that within two days the content of NE decreased in ventricular tissue.

Graph II

Myocardial Content of Catecholamine for Control and Burn Shocked Rats



In this study, it has been demonstrated that in burn shocked rats there is marked depletion in ventricular NE content. Coupling this data with the reports of increased urinary excretion of catecholamines following burn trauma, it appears that the catecholamine alterations observed in burn shocked animals closely resembles those changes seen in congestive heart failure.

One might ask the question, can the heart be depleted of Ne in the short time span of 6 to 8 hours as observed in this experiment? Coleman and Glaviano [10] (1963) have shown that rabbits in hemorrhagic hypotension have myocardial NE depletions within three hours. What might be the mechanism of this depletion? NE is generally accepted as the mediator of most sympathetic nervous activity, and it has been shown by Dahlstrom et al [11] (1965) that myocardial catecholamine seems to be localized almost exclusively in the terminal endings of the sympathetic nerves. Outshoorn and Vogt [42] (1952) demonstrated an increase in the NE content of coronary sinus blood of dogs in which they stimulated the cardiac sympathetic nerves. DeStefara [14] (1966) and Siegel [53] (1961) found myocardial catecholamine depletion after sympathetic nerve stimulation. Stitzel et al [58] (1965) working with rabbits more clearly delinated the pool of NE depleted during nervous stimulation. They found that the soluble rather than coarse or particulate fraction was the first to be depleted, and that this could be accomplished by stimulating the right cardiac accelerator nerve for as short a period as ten minutes.

It is possible that during the stress states of congestive heart failure, hemorrhagic hypovolemia and burn shock that the cardiac sympathetic innervation is depleted of NE simply by excessive stimulation. It has been shown by Kaho et al [31] (1961) that neither ventricular tachycardia nor ventricular fibrillation resulted in myocardial catecholamine depletion. However, in burns the resulting depletion may be dependent on the continued augmentation of myocardial contractility, which is necessary in the burn state, since the heart is attempting to maintain a body perfusion pressure in the face of a markedly lowered volume of venous return.

It is interesting to speculate why with an increase in circulating catecholamines as reflected by increased urinary output, the myocardium is not able to replenish itself with NE? Whey also did the myocardial conent of E increase in this study? It has been shown by Spann [55] (1966) that in guinea pigs with congestive heart failure after the infusion of NE that a smaller increment of this NE was found in the heart when compared with normals. They postulated that there must be some abnormal uptake or poor retention of NE in the failing heart. Chidsey [9] (1966) utilizing radioactively tagged NE found no abnormality in myocardial uptake of NE in the failing heart. Since it seemed that the uptake was apparently normal in the failing heart, Pool et al [46] (1967) investigated the synthesis of NE by the heart during experimental heart failure. They found a marked depletion of tyrosine hydroxylase activity. The biosynthesis

of NE proceeds through a series of steps from tyrosine to dopa to dopamine and finally to NE. The rate limiting step is the conversion of tyrosine to dopa which is catalyzed by tyrosine hydroxylase. The depression in the activity of this enzyme, tyrosine hydroxylase, is believed to result from prolonged sympathetic activity. Decreased activity of tyrosine hydroxylase maybe the etiology of myocardial NE depletion seen in burn shock. The augmentation of cardiac contractitity mediated by the sympathetic nervous system could possibly serve as the mechanism of initial depletion of NE, with an ultimate decrease in synthesis.

The possibility that a toxin released after the burn injury, plays a role in the depletion of myocardial NE must be considered. Fozzard [19] (1961) suggested that a toxin maybe involved in the decline of cardiac output in burn shock, but this was only a suggestion. No actual identification of a toxic substance affecting the myocardium of burned animals has been isolated. Rather than assume that a burn toxin is involved in the myocardial NE depletion it is more reasonable to conclude that this depletion maybe due to overactivity of the cardiac sympathetics. It is possible that in any severe stress situation in which continual augmentation of myocardial performance is required similar depletions of NE would be observed.

In this series of experiments, total depletion of NE was not found in the burn shocked rat's myocardium, and an increase in

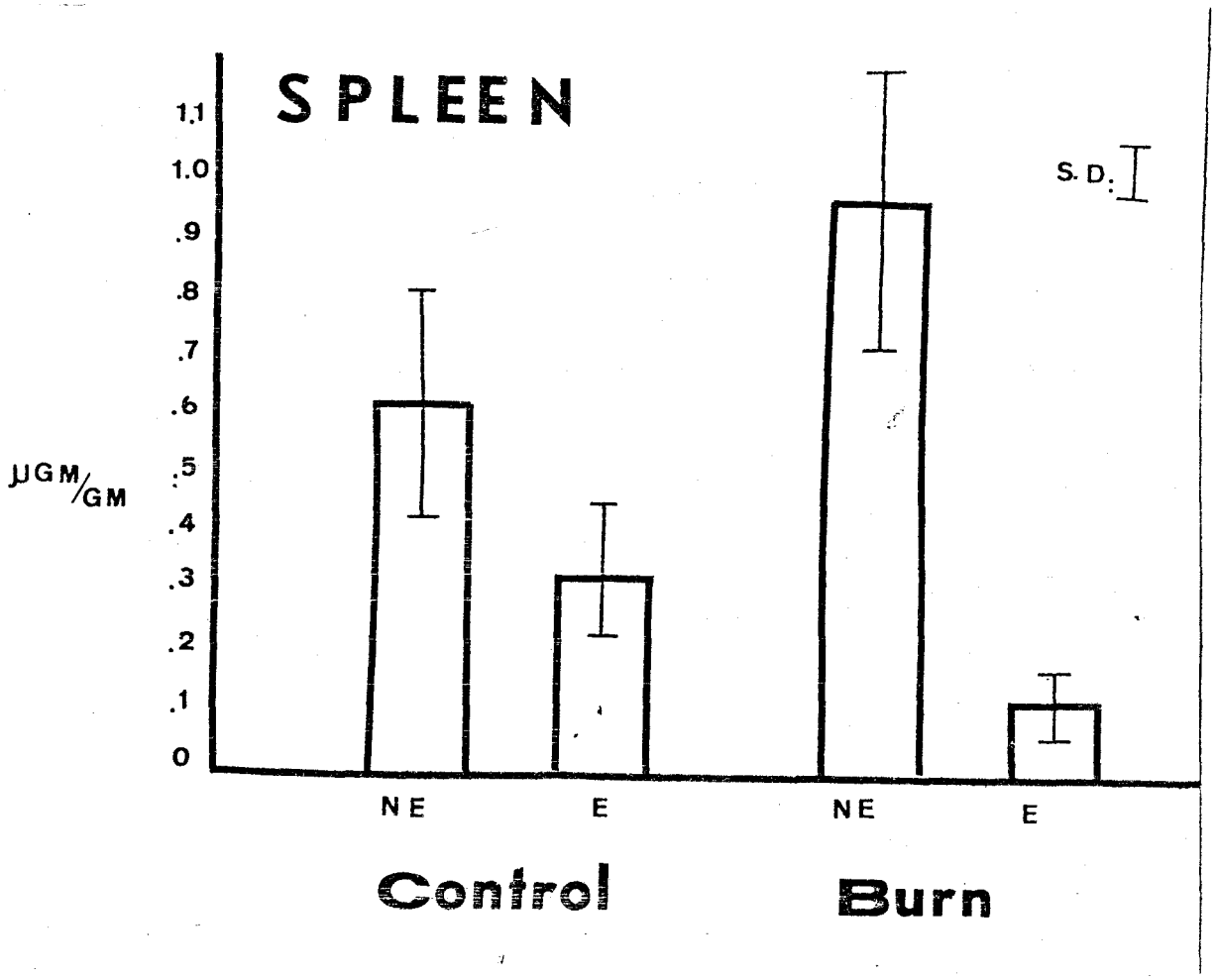
E was reported. It has been demonstrated by Inverson[29] (1963), in the rat that NE (H^3) is taken up by the heart, and Kopin[32] (1963), showed that more than 80% of the myocardial catecholamines resulted from synthesis. In the rabbit heart von Euler[17] (1965) found that both NE and E were taken up after chemical depletion of myocardial catecholamines by decaborane. In the heart of the burned rat the residual NE and the rise in E reported here may have been due to an uptake by the heart when presented with high circulating levels of NE and E.

It is interesting to note that Chidsey[9] (1965), was able to correlate increasing amounts of urinary catecholamine and progressive myocardial catecholamine depletion with the clinical state of the patient in heart failure. Tomomatsu[61] (1963) reported similarly, that as failure progressed, the myocardial catecholamine content decreased. Graph I demonstrates that the decrease in systolic blood pressure can be correlated with the level of myocardial norepinephrine content. A depression in systolic blood pressure was accompanied by a corresponding decline in myocardial NE. This would indicate that in the burn shock animal where many hemodynamic forces are active that myocardial NE stores are related to maintaining normal systolic pressure.

In hemorrhage shock Glaviano and Coleman[20] (1961) and Heft[28] (1965), reported a depletion of norepinephrine content especially in the spleen. Dahlstrom st al[11] (1965), confirmed the decreased

Graph III

Catecholamine Content of Control and Burn Shock Spleens



NE content of the post hemorrhagic spleen, and also demonstrated by means of fluorescent histology a diminished amount of fluorescence in the sympathetic splenic nerves of the shocked animal. In this study, it was observed that in the burn shocked rats the spleen underwent a depletion of E and an increase of NE content. In hemorrhagic hypovolemic shock the spleen is observed to undergo a marked constriction. In patients subjected to thermal injury, who had been treated with intensive fluid therapy, the spleens of those patients reaching necropsy were found to be markedly dilated and enlarged to twice the normal size, Wartman[65], (1960). Kabat[30] (1941) reported that in cats subjected to burn trauma the spleens were found to be dilated and enlarged. Muhkin[41] (1949) found that the spleens of dogs subjected to burns had lowered resistence and an increased amount of stored blood. It is obvious from these reports that in burn shock the spleen functions differently than it does in hemorrhagic shock. It is possible that the sympathetic outflow to the spleen in the burn state does not approach the magnitude seen in hemorrhage.

Spector et al [57] (1963), has shown that the spleen can synthesize 5 to 15% of its total catecholamine content per hour, and it was their conclusion that the splenic synthesis of catecholamine does not play a major role in replenishing the NE content. Undenfriend[62] (1963), reported similar findings. Whitky et al [66] (1961), in cats investigated the deposition of injected NE and E in the spleen. They

found that both NE and E were taken up in significant amounts when injected alone, but when given together, the uptake of NE was greater than E. The uptake of large amounts of circulating NE by the spleen could account for the increase seen in the burn shocked animal's spleen. The perferential uptake of NE over 6 to 8 hours could also have displaced E or replaced the utilized during that time. Dearnaley and Geffen[13] (1967), made an interesting observation on the splenic content of NE in spleens which they stimulated the sympathetic innervation with various trains of stimuli. They found that the NE content of the stimulated spleens increased if the analysis was done on a weight basis whereas it decreased in analyzed on a DNA basis. In their experiment the blood in the non-stimulated spleen in effect diluted the per gram splenic content of NE. This may play a role in the burn shocked spleen, but this is unlikely. As previously mentioned, the spleen following burns is usually dilated and not constricted.

The question might be raised, could the blood trapped in the spleen at any time contribute effectively to the splenic catecholamine content. The answer is no. The blood levels of NE and E in the normal state are approximately 1.5ugm/liter and 2.5ugm/liter respectively. In shock states these are increased approximately 10 x NE and 100 x E or .015ugm/ml NE and .25ugm/ml E. The spleens in the control and burn shock groups weighed from 1.2gm to .6gm, and the maximum amount of blood at any one time contained in the spleen is

is one milliliter. It is obvious that neither in the control nor in the burn group could the blood catecholamine content in the spleen contribute significantly to the tissue level.

Returning again briefly to the heart, can we attach any significance to the depletion of NE shown in this investigation. Spann et al [56] (1966), demonstrated the contractile state of the NE depleted myocardium is not altered nor were changes in the refractory period or electrical excitability of the heart observed. Thus NE stores are not necessary for an intact intrinsic myocardial contractile system. But as Siegel[54] (1961) pointed out, the release of NE is important in the position of the force or function curve of the heart. In the burn shock animal, the part played by the activity of the sympathetic innervation of the heart as an augmentor of myocardial function may well be a key factor. It's loss may be the mechanism of the shift to the right of the myocardial function curve in burn shocked dogs observed by Merrion[35], (1962). When faced with a decreased circulating volume, increased peripheral resistance, and an increase in blood viscosity, the depletion of norepinephrine and subsequent loss of sympathetic augmentation may herald the demise of the burned animal.

S U M M A R Y

In summary the foregoing experiments demonstrated that in the burn shocked rat the following catecholamine alterations take place.

1. There is a marked decrease in total myocardial catecholamine content. The NE content is decreased $P<.001$ within 6 to 8 hours after shock was induced. The E content is elevated fourfold in the burn shocked rats' hearts.

2. It appears that the systolic blood pressure possibly correlates with the myocardial content of NE. When systolic pressure is depressed, the myocardial NE is also decreased.

3. The spleens of burn shocked rats undergo alterations in catecholamine content which are different from those seen in the heart. Splenic NE increases marked $P<.05$ while E decreases. The total splenic catecholamine level is slightly elevated to 13% over control.

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